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Validation of a Rapid and Reliable Test for Diagnosis of Chagas' Disease by Detection of *Trypanosoma cruzi*-Specific Antibodies in Blood of Donors and Patients in Central America

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In this study we compared the performance of the Chagas Stat-Pak rapid immunochromatographic test with a standard enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of Chagas' disease in Central America. Out of 3,400 blood donor samples, 156 (4.6%) were positive in both assays. Three sera out of 2,084 samples from reference laboratories were negative with the rapid test but positive with the ELISA (99.8% agreement). Agreement of 100% between the two tests was observed with 339 additional sera from patients with cardiopathies and 175 sera from potential blood donors in emergency surgical cases occurring on weekends or at night. In conclusion, Chagas Stat-Pak showed 99.6% and 99.9% sensitivity and specificity, respectively, when assayed with 5,998 serum samples. It is a sensitive and specific alternative to the ELISA, as required in medical emergencies and blood screenings in Central America.

Chagas' disease, or American trypanosomiasis, is caused by the hemoflagellate protozoan *Trypanosoma cruzi* and is a major public health problem in Central America, where the estimated seroprevalence of infection is 7% (10, 13). *T. cruzi* is naturally transmitted to mammalian hosts through the urine and feces of infected hematophagous bugs or by blood transfusion or the ingestion of contaminated food; it may also be transmitted congenitally or through organ transplantation (10, 13). In Honduras, 20% of chronic cardiopathies are from chagasic patients, and 36% of pacemakers implanted in Guatemala and Honduras are for arrhythmias due to chagasic cardiopathy (7).

Chagas' disease is routinely diagnosed by commercial serological methods, such as enzyme-linked immunoassays (ELISAs), indirect immunofluorescence (IIF), and indirect hemagglutination, which use whole or semipurified extracts of the epimastigotes of *T. cruzi*. A considerable variation in the reproducibility and reliability of the results is observed with the three methods (1). The conventional serological tests (ELISA and IIF) are time-consuming (3 to 4 h) and consist of several steps, thus increasing the possibility of operational error (1, 4). An ELISA can be automated but at an increased cost that is beyond the reach of most laboratories in Central America. Therefore, many blood banks, routine diagnostics laboratories, and peripheral hospitals test a small number of samples per day, and, in general, these centers cannot afford the equipment and technicians needed to carry out the above-mentioned tests.

Nonconventional serological tests based on recombinant proteins or synthetic peptides have shown promising results for the diagnosis of *T. cruzi* infection (2, 3). These tests may, however, need to be adapted to local conditions. Umezawa et al. 2003 (11) have recently reported the combination of three *T. cruzi* recombinant antigens in a single ELISA, resulting in a multiantigen test that is very sensitive and specific for the diagnosis of Chagas' disease. On the basis of these results, a novel rapid immunochromatographic assay (Chagas Stat-Pak) was developed employing a defined mixture of these recombinant antigens (5). This test presents several advantages such as simplicity (one step), short execution time, absence of a need for special equipment or expertise, and, consequently, the possibility of use in the field at reduced cost. In addition, the option of storing the results indefinitely allows for subsequent confirmation by specialized staff.

With increasing interest in rapid diagnostic testing, labora-

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TABLE 1. Evaluation of Chagas Stat-Pak in different medical situations in Central America[a]

Country	Purpose of test	Total no. of samples	Chagas Stat-Pak (no. of samples)		ELISA (no. of samples)		Agreement[b] (%)
			Pos	Neg	Pos	Neg	
Honduras[c]	Confirmation	1,684	995	689	996	688	99.9
El Salvador[d]	Confirmation	200	67	133	69	131	97.1
Nicarágua[e]	Confirmation	200	12	188	12	188	100
Honduras[f]	Confirmation	339	97	242	97	242	100
Honduras[g]	Emergency	175	1	174	1	174	100
Honduras[h]	Exclusion	3,400	156	3,244	156	3,244	100
Total		5,998	1,328	4,670	1,331	4,667	99.8

[a] Abbreviations: Pos, positive; Neg, negative.
[b] Agreement between the results obtained with Chagas Stat-Pak and ELISA. In the present study the ELISA was taken as the gold-standard technique.
[c] Central Reference Laboratory for Chagas' disease and Leishmaniasis, Secretary of Health, Tegucigalpa, Honduras.
[d] Central Laboratory, Ministry of Public Health and Social Assistance, San Salvador, El Salvador.
[e] National Center for Diagnostic and Reference, Ministry of Health, Managua, Nicaragua.
[f] INS: National Thorax Institute, Tegucigalpa, Honduras.
[g] CRH-CENASA: Red Cross Laboratory-National Blood Center, Tegucigalpa, Honduras.
[h] Blood banks. See Table 2 for detailed descriptions of blood banks in Honduras.

tories are reviewing their ordering options for immunoassay kits to include in their routine protocols. Here we present the evaluation of Chagas Stat-Pak performance in a large field study in Central America. The test was used in the following situations: prescreening of random blood donors, selection of blood bags for transfusion in emergency surgical cases, and confirmation of diagnosis in cases of cardiopathy and other conditions. This study shows the advantages of employing this diagnostic tool in regions where Chagas' disease is endemic.

MATERIALS AND METHODS

Immunochromatographic assay. Chagas Stat-Pak (Chembio Diagnostic Systems, Medford, NY) is a rapid immunochromatographic screening test for detection of anti-*T. cruzi* antibodies in whole blood, serum, or plasma (5). It employs a unique combination of *T. cruzi* recombinant antigens (B13, 1F8, and H49/JL7) (described in reference 11), which are bound to the membrane, and a specific antibody-binding protein, which is conjugated on dye particles. As the test sample flows laterally through the membrane, the antibody-binding protein-dye conjugate binds to human immunoglobulins in the sample. A drop of serum (5 μl) is placed in the sample well at the holder, and buffer provided with the kit is added. After 5 to 15 min, the mixture of serum plus buffer migrates to the top of the device. The end of the reaction is indicated by a colored line on the top (positive control). The presence of anti-*T. cruzi* antibodies in the sample produces a pink/purple line (positive), whereas in its absence no line appears in the reaction zone (negative). A second pink/purple line in the control zone confirms that the reaction was completed and that the test is, hence, validated. Reading of the results on the appropriate region of the device is performed by recording the absence of any line as negative and a strong or weak line as positive.

ELISA. All serum samples were also analyzed with a commercial ELISA kit (Chagatest recombinante; Wiener, Argentina) used routinely in Honduras and El Salvador. In Nicaragua, the National Center for Diagnostic and Reference employed an in-house ELISA, prepared with antigens from a local *T. cruzi* strain following the technique described by Voller et al. (12).

Study populations. Human sera were obtained from random blood donors and patients with clinical symptoms consistent with Chagas' disease. The study was approved by the institutional review boards of the Secretary of Health, Honduras, and the human experimentation guidelines of this institution were followed. The 5,998 serum samples used in this study consisted of the following: (i) samples collected from candidates for blood donation (n = 3,400) at seven blood banks in Honduras from January to December 2000 (Tables 1 and 2); (ii) samples from blood donors (n = 175) tested only by Chagas Stat-Pak before transfusion, in emergencies occurring on weekends or at night at the Red Cross Laboratory in the National Blood Center of Honduras, Tegucigalpa (Table 1); (iii) samples from patients (n = 339) who consulted at the National Thorax Institute of Honduras and were diagnosed with cardiopathy (Table 1); and (iv) samples received by reference diagnostic laboratories in Honduras (n = 1,684), El Salvador (n = 200), and Nicaragua (n = 200) for exclusion or confirmation of Chagas' disease (Table 1).

Each sample was first tested by the staff of each laboratory with the Chagas Stat-Pak kit and afterwards by ELISA. Readings were recorded by each observer, and in the case of doubtful results, a second observer was consulted. In this study, the ELISA was selected as the gold-standard test. The values for sensitivity and specificity and for agreement between Chagas Stat-Pak and ELISA were calculated as described previously (3).

RESULTS

In a previous study, the Chagas Stat-Pak test showed 100% sensitivity when assayed with sera collected in Honduras (5). To confirm and extend these results, a larger number of serum samples (n = 2,084) was analyzed with Chagas Stat-Pak and ELISA in three reference laboratories in Honduras (n = 1,684), Nicaragua (n = 200), and El Salvador (n = 200) (Table 1). From this total, 1,077 and 1,007 serum samples, respectively, were positive and negative by ELISA, while 1,074 and 1,010 samples, respectively, were positive and negative by Chagas Stat-Pak. This means that three sera with anti-*T. cruzi* antibodies were recognized by ELISA but were not detected by the Chagas Stat-Pak. These three sera with discrepant results were tested also by IIF and by indirect hemagglutination, with positive results that were in accord with the ones obtained by the Wiener ELISA. The agreement between the two tests was 100% with sera from Nicaragua and 99.9% and 97.1% with sera from Honduras and El Salvador, respectively.

The rapid and accurate diagnosis of Chagas' disease is imperative in order to provide patients at the National Thorax Institute of Honduras with appropriate treatment and supportive therapy. Sera from 339 patients who consulted for the first time at the National Thorax Institute and were diagnosed as having cardiopathy were assayed using Chagas Stat-Pak (Table 1). Ninety-seven patients were positive. The same result was confirmed for all samples with ELISA, with 100% agreement.

Chagas Stat-Pak was tested as a possible tool for use in emergencies in regions where Chagas' disease is endemic, where a donor should be evaluated quickly before his/her blood is delivered into a severely disabled patient. This situation is common in remote cities, where few facilities are avail-

TABLE 2. Evaluation of Chagas Stat-Pak in Honduran blood banks[a]

Center	Prevalence[b] (%)	Total no. of samples	Chagas Stat-Pak (no. of samples)		ELISA (no. of samples)		Agreement[c] (%)
			Pos	Neg	Pos	Neg	
HIT	0.3	240	0	240	0	240	100
HSL	0.7	190	1	189	1	189	100
CRH-CERESA	1.1	1,172	42	1,130	42	1,130	100
HGSF	1.5	1,240	64	1,176	64	1,176	100
HST	2.1	252	15	237	15	237	100
HJS	5.2	132	12	120	12	120	100
HEA	4.5	174	22	152	22	152	100
Total		3,400	156	3,244	156	3,244	100

[a] Abbreviations: Pos, positive; Neg, negative; HIT, Hospital of Tela Integrado, Tela, Atlântida; HSL, Hospital San Lorenzo, San Lorenzo, Valle; CRH-CERESA, Red Cross Laboratory-Regional Blood Center, San Pedro Sula, Cortes; HGSF, Hospital General San Felipe, Tegucigalpa; HST, Hospital Santa Teresa, Comayagua; HJS, Hospital Jesús Subirana, Yoro; HEA, Hospital Enrique Aguillar, La Esperanza, Intibucá.
[b] Data from the Secretary of Health of Honduras, obtained from 1998 to 2002.
[c] Agreement between the results obtained with Chagas Stat-Pak and ELISA. In the present study the ELISA was taken as the gold-standard technique.

able and where there are limited blood reserves. In such situations, fast methods should be used, or the patient may die. Using only Chagas Stat-Pak, the Red Cross Laboratory in Tegucigalpa (Honduras) tested 175 blood bags in emergencies that required surgical procedures during weekends or at night. Results were negative in 99.4% of the sera ($n = 174$); hence, blood was immediately transfused to the recipients. When Chagas Stat-Pak showed a positive result, the blood was refused, and blood from another negative donor was used instead. After recipients were transfused in emergencies with blood tested with Chagas Stat-Pak, the sera were tested with routine ELISA, which yielded the same results as the rapid test; i.e., 174 sera tested negative, and the single positive case detected by Chagas Stat-Pak was confirmed positive by ELISA (Table 1).

The specificity of Chagas Stat-Pak was estimated in blood donor populations with both a high and low prevalence of Chagas' disease and compared with that of an ELISA using the same specimens (Table 2). A total of 3,400 candidates for blood donation were screened by both tests in seven Honduran blood banks, with 156 positive (4.6%) by Chagas Stat-Pak. All of them were confirmed by ELISA, which is used routinely, with 100% agreement. There were no differences in the results among the seven blood banks that took part in this study for either test.

DISCUSSION

Most countries in Central America have relatively small territorial extensions and a high prevalence of *T. cruzi*-infected individuals (7, 10, 13). In general, laboratories for the diagnosis of infectious diseases are small, have few resources, and perform a limited number of tests per day. These laboratories, however, are obliged to include diagnosis of anti-*T. cruzi* antibodies in candidates for blood donation because of specific legislation. In addition, in 1997 "The Central American Initiative for the Control of Vectorial and Transfusional Transmission of *T. cruzi*" was launched, aiming at the elimination of insect vectors and the screening of all blood donors (13). There is, therefore, a need for a rapid and reliable test for Chagas' disease to be used in these circumstances, and such a test should be commercially available for immediate use. In addition, the test should have high specificity and sensitivity and be validated with sera from Central America in order to be able to diagnose individuals infected with *T. cruzi* strains circulating in these regions (6, 13, 14). Finally, such a test should be suitable for use with a small number of samples per day. Studies performed with Chagas Stat-Pak and published recently (5) suggest that this test fulfills all these requirements.

The present study was designed to test this hypothesis. For this purpose, almost 6,000 serum samples, covering three Central American countries, were tested. The samples were obtained from reference laboratories, blood banks, and hospitals. Results showed that the Chagas Stat-Pak performed well with the 5,998 serum samples, of which only 3 tested negative compared with the ELISA (0.05% disagreement). There was perfect agreement with all the samples from the seven blood banks included in the study. These findings allow us to recommend Chagas Stat-Pak as a second test in blood banks and as a single test in emergencies. The performance of the test in patients presenting cardiopathies also correlated closely with the results obtained by ELISA. Other commercial tests are available, such as PaGia (8) and Innolia (9), but they need to be performed in laboratories, demand more skill, and involve more steps and, hence, would not fulfill the requirement of a quick and easy test, as the Chagas Stat-Pak tested in this study does.

We conclude that Chagas Stat-Pak meets all the requirements for use in Central American blood banks and laboratories for confirmation of the infection, as well as in seroepidemiological studies. In addition, the test can be performed without the need for special skills and can be used for a small number of samples. Although the cost of the Chagas Stat-Pak rapid cartridges is higher than that of a single ELISA, Chagas Stat-Pak is more economical if the costs associated with laboratory personnel, quality control, and reagent storage are taken into account.

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